Evaluating Phytoremediation of Lead-Contaminated Soils in Lo‘i Agriculture

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Abstract

Traditional irrigated pondfields, known as lo‘i agriculture, are one of the most iconic forms of Hawaiian food cultivation. The practice of lo‘i agriculture not only produces the ancestral food kalo (*Colocasia esculenta*), but also establishes key cultural connections to land. Soil lead (Pb) contamination, however, poses a serious hazard to the many people perpetuating this important practice. While phytoremediation (i.e., growing plants to remove contaminants from the soil) is often implemented to address soil contamination issues, no study has yet to test if phytoremediation is effective in lo‘i systems. Thus, the goals of this study were to: (*i)* investigate whether certain plants are more effective at uptaking bioavailable Pb in lo‘i soils, (*ii*) determine which parts of each plant (roots or shoots) accumulate the most Pb, (*iii*) and extrapolate the amount of rounds of phytoremediation needed to reduce soil Pb concentrations to a safe level. Three different phytoremediation plantings: (1) the native wetland plant ‘ae‘ae (*Bacopa monnieri*), (2) the widely studied plant Indian mustard (*Brassica juncea*), and (3) a control containing naturally established weedy species (Honohono grass - *Commelina diffusa,* Mexican primrose - *Ludwigia octovalvis*, and nutsedge - *Cyperus rotundus*) were grown *in situ* at a Pb contaminated lo‘i site until full maturation. Following one round of phytoremediation, the ‘ae‘ae roots contained significantly higher Pb concentrations than any other plant biomass component (Tukey HSD test, $P < 0.001$). Furthermore, ‘ae‘ae plantings had a higher total Pb uptake than Indian mustard plantings (Tukey HSD test, $P < 0.001$). There were no significant differences, however, between pre- and post-planting soil Pb concentrations. Based on the estimated mass of Pb at the lo‘i site, ~1,000-120,000 rounds of phytoremediation would be required to reduce soil Pb concentrations to a safe level (0-75 mg/kg Pb), translating to 100-18,000 years of remediation time. In conclusion, implementing any of the tested plantings alone would not be practical to reduce Pb contamination at the lo‘i site. Future efforts will need to consider other plants or alternative methods such as a combination of soil removal and phytoremediation to address Pb contamination issues in lo‘i agriculture.

Keywords: Hawai‘i, heavy metals, restoration, Indian mustard, ‘ae‘ae, taro

Table of Contents

List of Figures

List of Tables

Motivation

Introduction

The cultivation of kalo (*Colocasia esculenta*) is one of the most important practices in Hawaiian culture[1,2,3,4]. Ancestral foods such as kalo not only comprise the basis of Hawaiian diet, medicine, ceremony, and lifestyle, but more importantly, embody a sacred relationship in Hawaiian society[5]. According to moʻolelo (Hawaiian stories and sources of knowledge), kalo is directly traced to the origins of the Hawaiian people and is regarded as the esteemed elder brother to the kānaka maoli (Native Hawaiians)[3,6]. Thus, the act of cultivating kalo serves to both nourish the Hawaiian people, as well as establish key cultural values and connections between people and the land.

Importance of Loʻi Agriculture

As the most iconic method of kalo cultivation in Hawaiʻi, loʻi agriculture is found in every major island of the archipelago[3]. Loʻi systems, at their most basic form, consist of a flooded pondfield with irrigation from a natural source. Loʻi farmers additionally implement various structures and specific planting techniques to accommodate unique island characteristics (e.g. steep mountain slopes, valley floors, perennial springs, marshes, etc.), making these systems successful in many diverse environments throughout Hawaiʻi[4]. Prior to Western contact in 1778, an estimated 31,688-48,627 acres of land in Hawaiʻi could have been used for loʻi agriculture[7,8]. In 2013, however, loʻi agriculture only encompassed 370-600 acres (≤0.1% of total agricultural land in Hawaiʻi)[9]. Many factors have contributed to this vast reduction in loʻi agriculture, including: loss of Hawaiian people and knowledge, diversion of water sources, introduction of more economically valued crops (e.g. sugarcane and rice), and land conversion to housing and other uses[,9,,10,11]. While these factors still impede loʻi agriculture today, many kānaka maoli and local families are continuing to revitalize this important practice.

Soil Lead Contamination

Soil contamination of heavy metals such as lead (Pb) is a serious concern for restoring loʻi systems. The Hawaiʻi State Department of Health (HDOH) identifies that urban areas, particularly those near busy roadways and old buildings, are most commonly contaminated with Pb[12]. In fact, Sutherland and Tolosa (2001) showed that Pb enrichment can occur up to 50m away from roadways in an urban Honolulu watershed[13]. Since many historical loʻi areas have been converted to urbanized land uses[9,10,11], kalo farmers and cultural practitioners today are operating or seeking to operate in more urbanized and potentially contaminated areas. For example, the non-profit organizations Papahana Kuaola and Kauluakalana conduct their loʻi agriculture on former dumping sites located in residential areas.

Lead toxicity can lead to major health problems such as seizures, cardiovascular problems, and reproductive complications[12]. Furthermore, excessive Pb exposure in children can cause impaired development, reduced intelligence, short-term memory loss, and disabilities in learning and coordination[14]. Since there is no safe level of Pb for human consumption[15], ingestion of contaminated kalo poses a major health hazard. Islam et al. (2016) showed that kalo is capable of accumulating significant levels of Pb (7748 mg Pb/kg shoot biomass) when grown in highly contaminated soils (1200 mg Pb/kg)[16]. Moreover, physical exposure or accidental ingestion of Pb contaminated soil is also a major hazard[12] for farmers, cultural practitioners, and especially families who engage in lo‘i restoration. To address this issue and support the restoration of lo‘i agriculture, this study evaluated the potential of *phytoremediation* to remediate lo‘i soils contaminated with Pb.

Background

Phytoremediation

Phytoremediation is a popular strategy used to address soil contamination issues throughout the world[17,18], and is broadly defined as the intentional cultivation of plants to clean contaminated environments[17]. Among the different mechanisms of phytoremediation (e.g., phytostabilization, rhizofiltration, phytovolatilization)[18], this study specifically focused on the process of phytoextraction to remediate Pb contamination of loʻi soils. Phytoextraction is the cultivation of certain plants (i.e., hyperaccumulators) to uptake contaminants including heavy metals from the soil and store it within their biomass[17,18]. Transpiration drives this process, as heavy metals in dissolved ionic form enter through the roots and translocate to the shoots via the xylem[19].

The majority of Pb found in the soil, however, is unavailable for plant uptake due to complexation with carbonates, oxides, phosphates, hydrazides, and organic matter[20]. In fact, only about 0.1-2.2% of Pb in soil is typically bioavailable (in ionic and exchangeable forms)[21,22]. Thus, ideal phytoextracting plants need to be able to mobilize and accumulate high amounts of Pb from the soil[17,18,19]. Ideal plants also need to tolerate difficult growing conditions (e.g., soil pH, salinity, water content), produce high biomass, and store contaminants in accessible parts of the plant (e.g. leaves and shoots)[17,18,19].

Phytoremediation Plants

Indian mustard (*Brassica juncea*) has been widely recognized to phytoextract heavy metals such as lead, mercury, arsenic, and cadmium[18,20,22,]. Not only can this plant tolerate a wide range of soil acidity (pH 5.1-7.1), but it can also achieve almost twice as high Pb concentration within the plant biomass as compared to the surrounding soil Pb concentration[18]. In a previous study, Indian mustard accumulated on average 1,091 mg/kg Pb (dry weight) over a two year period, which reduced the average soil Pb concentration by 25% (635 mg/kg to 478 mg/kg) at a contaminated site in Connecticut[18].

In Hawaiʻi, only a few phytoremediation studies have addressed soil contamination of heavy metals[23,24]. Moreover, no studies have yet investigated the effectiveness of phytoremediation in loʻi systems. Given the unique physical characteristics of loʻi systems (i.e., flooded/anaerobic conditions, high clay content), only a few plants are likely suitable for phytoremediation. ʻAeʻae (*Bacopa monnieri*), however, is a native wetland plant that can thrive in loʻi systems. In fact, many loʻi farmers consider this plant a “weed”. Although few studies have tested the ability of ʻaeʻae to phytoextract heavy metals in soils, Sinha (1999) indicated that ʻaeʻae is at least capable of accumulating heavy metals and storing it within their root biomass[25].

Objectives

The overall objective of this study was to assess if phytoremediation is a potential solution for remediating soil Pb contamination in lo‘i agriculture. Thus, the goals of this study were to: (*i)* investigate whether certain plants are more effective at uptaking bioavailable Pb in lo‘i soils, (*ii*) determine which parts of each plant (roots or shoots) accumulate the most Pb, (*iii*) and extrapolate the amount of rounds of phytoremediation needed to reduce soil Pb concentrations to a safe level.

Approach

Study Site

This study consisted of conducting an *in situ* phytoremediation experiment at a lo‘i site located at the base of Ulupō heiau (sacred Hawaiian place of worship) in the ahupua‘a (traditional land division) of Kailua on the Island of O‘ahu (Figure 1). As a former dumping ground, this recently established lo‘i site (≤20 years old) faces serious concerns of soil Pb contamination. Preliminary soil Pb levels of this area average 161.2 ± 29.4 ppm (n=18)[26], yet typical soil Pb levels throughout Hawai‘i range from 10-75 ppm[12]. Nine individual lo‘i plots fed from the same nearby spring comprise this study site, and the soil series of this area is the Pohakupu silty clay loam (Fine, parasesquic, isohyperthermic Oxic Humustepts; bulk density = 1.3 g/cm^3)[27]. Historically, Ulupō heiau was a well stacked rock structure used in ceremonies by kānaka maoli for the abundant production of food. Today, much of the heiau structure has changed, however, the non-profit organization Kauluakalana stewards this sacred site. Furthermore, because the mission of Kauluakalana is to reconnect people to land and place, many students and families regularly engage in lo‘i work at this site.

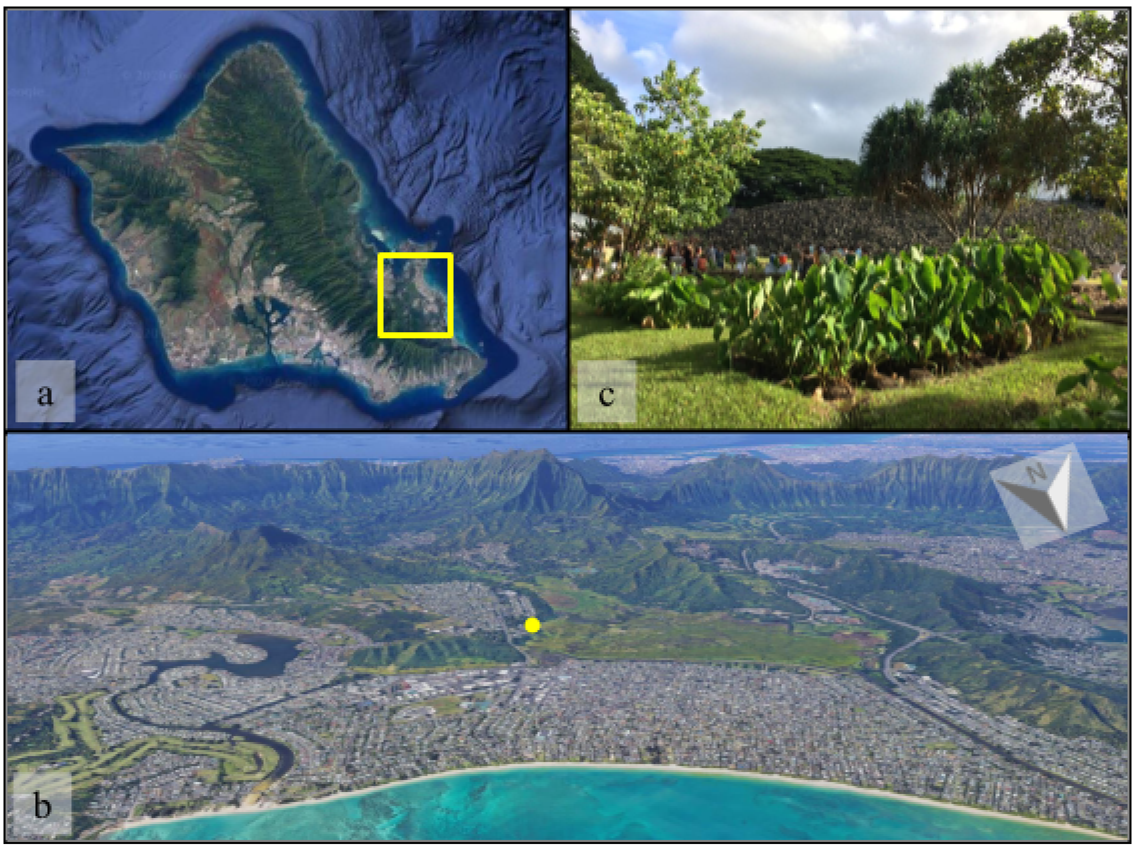

Figure 1. The study site of this capstone project. (a) The study site is located in Kailua (yellow box) on the Island of O‘ahu. (b) 3D Google Maps image of Kailua. The yellow dot marks the location of Ulupō heiau. (c) The lo‘i site planted with kalo (*Colocasia esculenta*) located at the base of Ulupō heiau (rock structure in the background).

Experimental Design

Three different phytoremediation plantings were grown in individual lo'i plots of the study site (n=3 lo'i plots per planting; Figure 2). These plantings were (1) the native wetland plant 'ae'ae, (2) the widely studied plant Indian mustard, and (3) a control containing a mix of naturally established weedy species (Honohono grass - *Commelina diffusa*, Mexican primrose - *Ludwigia octovalvis*, and nutsedge - *Cyperus rotundus*). Plantings were assigned to lo'i plots in a non-randomized fashion because the selected plants naturally grow in different soil conditions (e.g., 'ae'ae is a wetland plant and Indian mustard is dryland vegetable). To maximize plant growth, 'ae'ae plants were grown in fully saturated lo'i plots (i.e., watered by flooded irrigation), while Indian mustard and control plants were grown in naturally irrigated lo'i plots (i.e., watered by rain and occasional spring upwelling). Indian mustard and 'ae'ae plants were propagated by evenly dispersing seeds (5,000 seeds per lo'i plot; Figure A1) and plant fragments (~30 lbs of 'ae'ae per lo'i plot; Figure A2) respectively, by hand.

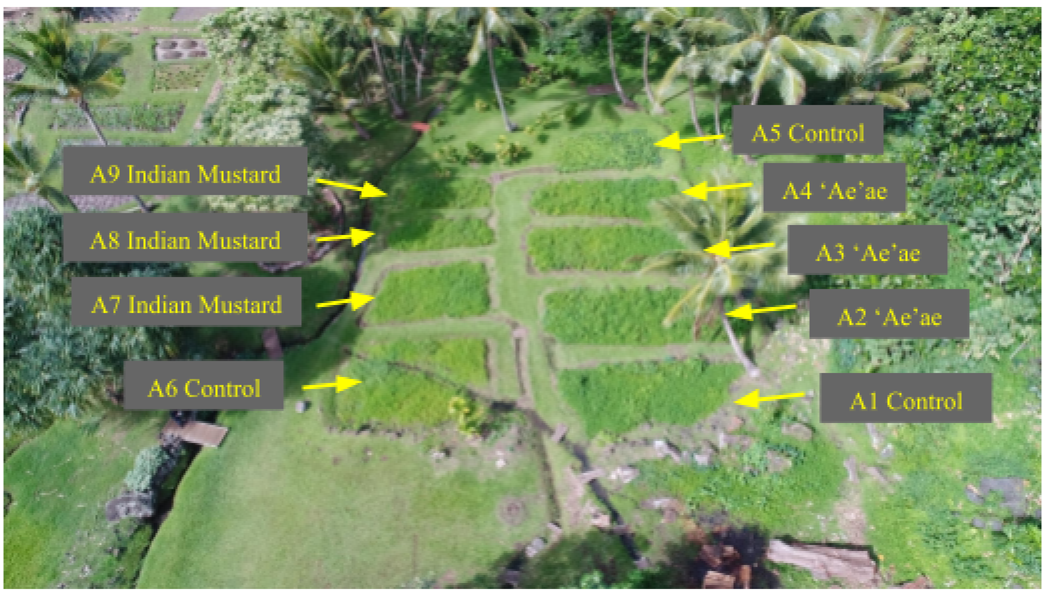

Figure 2. Phytoremediation plantings assigned to their respective lo'i plot (A1-A9). Three lo'i plots were planted with Indian mustard, three lo'i plots are planted with 'ae'ae, and three lo'i plots are control plots.

Collecting and Analyzing Biomass Samples

After plants reached full growth (Indian mustard and control plants: eight weeks after planting, 'ae'ae: five months after planting; Table A1), plants were removed from lo'i plots. This process required seven steps (Figure 3). (1) First, each lo'i plot was divided into quadrants. (2) In each quadrant, a random 0.5x0.5m square area was selected (using a 0.5x0.5m PVC square). (3) Then, all plants within the 0.5x0.5m square were harvested and excess soil on the roots was washed off with water. (4) Harvested plants were separated by roots and shoots, (5) oven-dried for at least 72 hours (60°C), and (6) weighed to determine average root and shoot biomass growth (kg/0.25m^2/day) for each of the phytoremediation plantings. (7) Lastly, dried root and

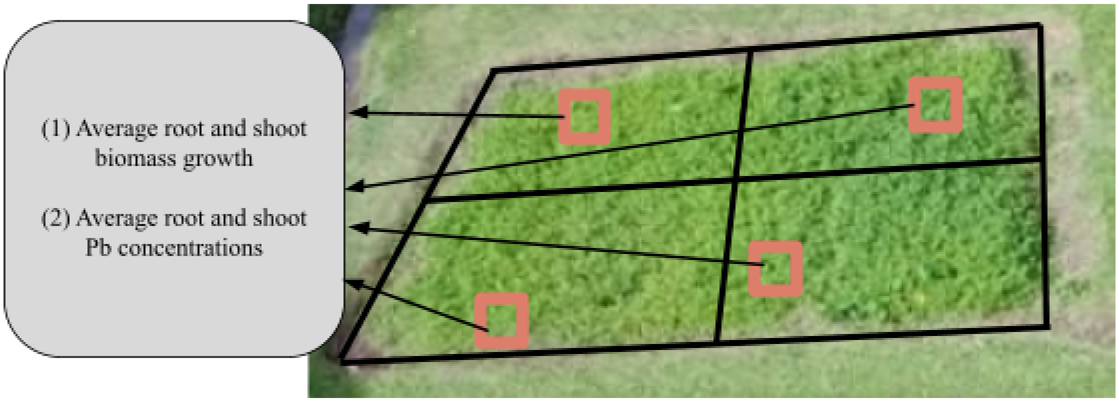

Figure 3. Example of removing plants from a lo‘i plot using the described plant removal design (superimposed). Red squares represent random 0.5x0.5m square areas. Harvested plants were used to obtain the (1) average dry root and shoot biomass growths and the (2) average root and shoot Pb concentrations.

shoot biomass samples were sent to a laboratory (ALS Global) to obtain Pb concentrations via homogenization and Inductively Coupled Plasma/Mass Spectrometry[28].

For all biomass Pb concentration data, a two way ANOVA (Type III) was performed using R software[29] to assess statistical differences in biomass Pb concentrations. Note, four outliers were removed in order to meet the ANOVA requirements of normal distribution (Table A3) and homogeneity (Table A4) within the dataset. A Tukey Honest Significant Difference (Tukey HSD) Test was additionally performed to identify which phytoremediation plantings and biomass parts had significantly higher Pb concentrations.

Root and shoot Pb uptakes (mg Pb/0.25m^2/day) of each phytoremediation planting were also calculated using the average biomass growths and the average biomass Pb concentrations (Figure 4). Furthermore, these root and shoot uptake values were summed to obtain the total Pb uptake of each phytoremediation planting (mg Pb/0.25m^2/day). To compare any statistical differences in total Pb uptake, a one way ANOVA (Type III) was performed. Note, three outliers were removed in order to meet the ANOVA requirements of normal distribution (Table A7) and

(1) $Avg\ root\ biomass\ growth\ (kg/0.25m^2/day) \times Avg\ root\ Pb\ concentration\ (mg\ Pb/kg) = Root\ Pb\ uptake\ (mg\ Pb/0.25m^2/day)$

(2) $Avg\ shoot\ biomass\ growth\ (kg/0.25m^2/day) \times Avg\ shoot\ Pb\ concentration\ (mg\ Pb/kg) = Shoot\ Pb\ uptake\ (mg\ Pb/0.25m^2/day)$

(3) $Root\ Pb\ uptake\ (mg\ Pb/0.25m^2/day) + Shoot\ Pb\ uptake\ (mg\ Pb/0.25^2/day) = \mathbf{Total\ Pb\ uptake\ (mg\ Pb/0.25m^2/day)}$

Figure 4. Calculating root, shoot, and total Pb uptakes (mg Pb/0.25m^2/day) of each phytoremediation planting.

homogeneity (Table A8) within the dataset. Lastly, a Tukey HSD Test was performed to identify which phytoremediation plantings had significantly higher total Pb uptakes.

Collecting and Analyzing Soil Samples

For each loʻi plot, five soil samples were randomly collected before planting (pre-planting) and after harvesting (post-planting). Note, only the top 10 cm of soil were extracted using a pvc pipe (≥100 g of soil per sample). After collection, all soil samples were oven dried for 72 hours (60°C), and then submitted to a laboratory (ALS Global) to obtain Pb concentrations via Inductively Coupled Plasma/ Mass Spectrometry[28]. To see which plant was more effective at reducing loʻi soil Pb concentrations, differences in pre- and post-planting soil Pb concentrations were compared using a two way ANOVA on R software[29].

Estimating Phytoremediation Needs

Finally, this study extrapolated the number of phytoremediation rounds needed to lower soil Pb concentrations of Ulupō loʻi plots to a safe level (0-75 mg/kg Pb)[12]. One phytoremediation round is the time from plant propagation to removal. This estimation required a few steps. First, the total mass of Pb in each loʻi plot was calculated using the measured area, bulk density, and average soil Pb concentrations of each loʻi plot (Figure 5).

(1)  $= Area\ of\ lo'i\ (m^2)$

(2) $Area\ of\ lo'i\ (m^2) \times Soil\ depth\ (0.10m) = Soil\ volume\ (m^3)$

(3) $Soil\ volume\ (m^3) \times Bulk\ density\ (1300\ \ kg/m^3) = Soil\ mass\ (kg)$

(4) $Soil\ mass\ (kg) \times Avg\ soil\ Pb\ concentration\ (mg\ Pb/kg) = \boldsymbol{Mass\ of\ Pb\ (mg)}$

Figure 5. Calculating mass of Pb (mg) in each loʻi plot.

By dividing the total mass of Pb in each loʻi plot with the total Pb uptake of the respective phytoremediation planting (Figure 6), the number of phytoremediation rounds needed to completely remove Pb from each loʻi plot was estimated. Note, total Pb uptakes were scaled to the area of their respective loʻi plots and to the duration of one phytoremediation round. The number of years needed for complete Pb removal was also estimated using the time of one phytoremediation round (Table A1). Lastly, these steps were repeated (Figure 7) to estimate the number of phytoremediation rounds and years needed to reach the upper threshold of normal soil Pb concentrations (75 mg/kg Pb)[12].

(1) $Mass\ of\ Pb\ (mg)/(Total\ Pb\ uptake\ (mg\ Pb/0.25m^2/day) \times Area\ of\ lo'i\ (m^2) \times Time\ of\ one\ phyto.round\ (day)) =$
$\boldsymbol{Number\ of\ phytoremediation\ rounds\ (complete\ Pb\ removal)}$

(2) $Number\ of\ phytoremediation\ rounds \times Time\ of\ one\ phyto.round\ (year) =$
$\boldsymbol{Time\ needed\ for\ comlete\ Pb\ removal\ (year)}$

Figure 6. Calculating the number of phytoremediation rounds and time (years) needed to completely remove Pb from the lo‘i soil.

(1) $Soil\ mass\ (kg) \times soil\ Pb\ concentration\ (75\ mg\ Pb/kg) = Mass\ of\ Pb\ (mg)$

(2) $Mass\ of\ Pb\ (mg)/(Total\ Pb\ uptake\ (mg\ Pb/0.25m^2/day) \times Area\ of\ lo'i\ (m^2) \times Time\ of\ one\ phyto.round\ (day)) =$
$Number\ of\ phytoremediation\ rounds\ (removal\ of\ 75\ mg\ Pb/kg)$

(3) $Number\ of\ phyto.rounds\ (complete\ Pb\ removal) - Number\ of\ phyto.rounds\ (removal\ of\ 75\ mg\ Pb/kg) =$
$\boldsymbol{Number\ of\ phytoremediation\ rounds\ (removal\ down\ to\ 75\ mg\ Pb/kg)}$

(4) $Number\ of\ phytoremediation\ rounds\ (removal\ down\ to\ 75\ mg\ Pb/kg) \times Time\ of\ one\ phyto.round\ (year) =$
$\boldsymbol{Time\ needed\ for\ removal\ down\ to\ 75\ mg\ Pb/kg\ (year)}$

Figure 7. Calculating the number of phytoremediation rounds and time (years) needed to remove Pb from the lo‘i soils down to 75 mg Pb/kg.

Results

Plant Biomass Growth

Out of the three phytoremediation plantings, control plants had the highest root and shoot biomass growth (Figure 8; Table A2). In fact, shoots of the control plants grew an order of magnitude higher than any other plant biomass. Indian mustard, in contrast, had the lowest root and shoot biomass growth.

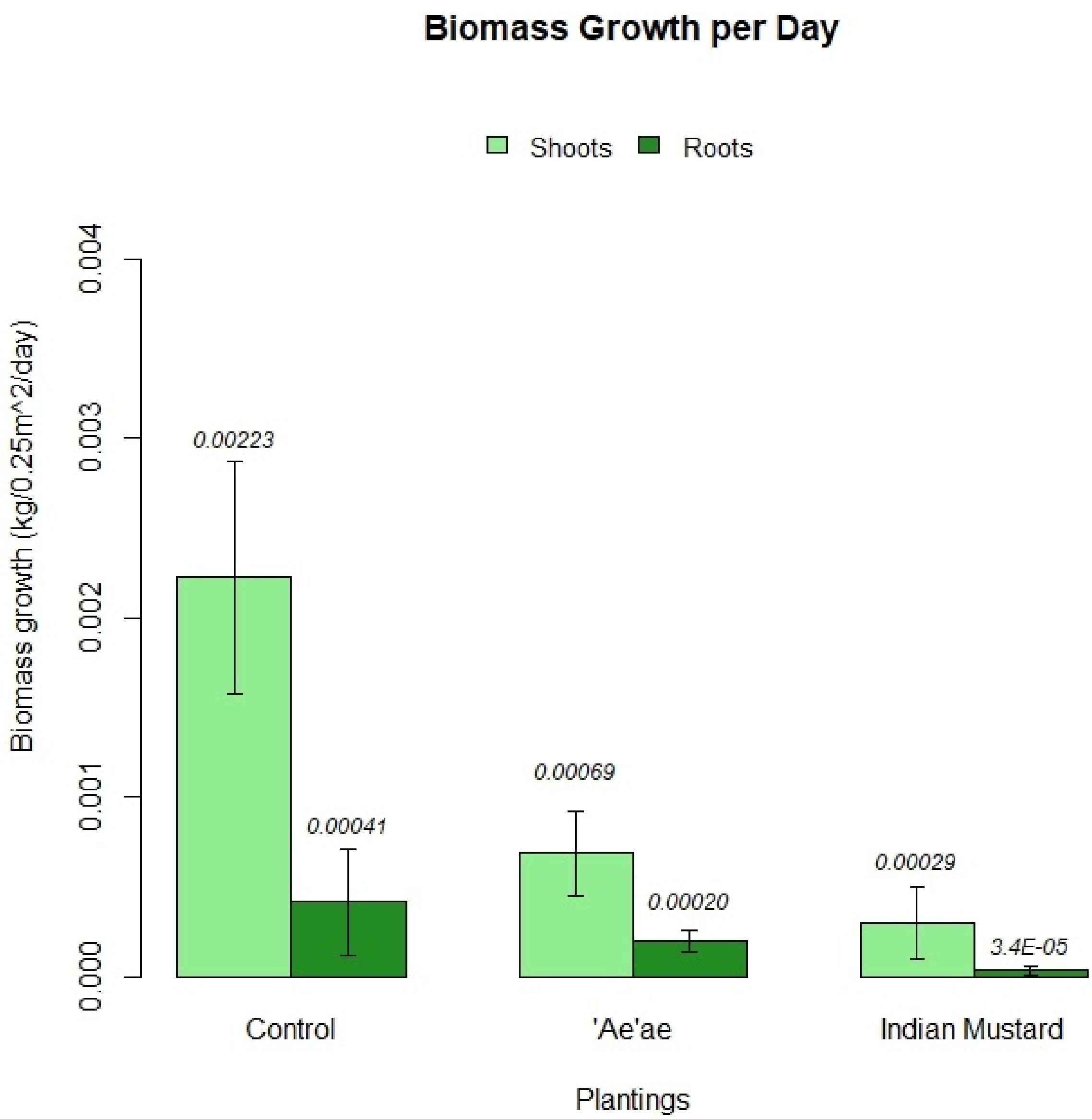

Figure 8. Average root (dark green) and shoot (light green) biomass growth (kg/0.25m^2/day) of each phytoremediation planting with standard deviation bars. Control plants had the highest root and shoot growth than any other plant.

Biomass Pb Concentrations

Biomass Pb concentrations significantly differed among the different combinations of phytoremediation plantings and biomass parts ($P < 0.001$; Table 1). In particular, ‘ae‘ae roots contained significantly higher Pb concentrations ($P < 0.001$) than any other plant biomass (Figure 9; Table A5). In contrast, Pb concentrations of Indian mustard root and shoot biomass did not differ from control root and shoot biomass.

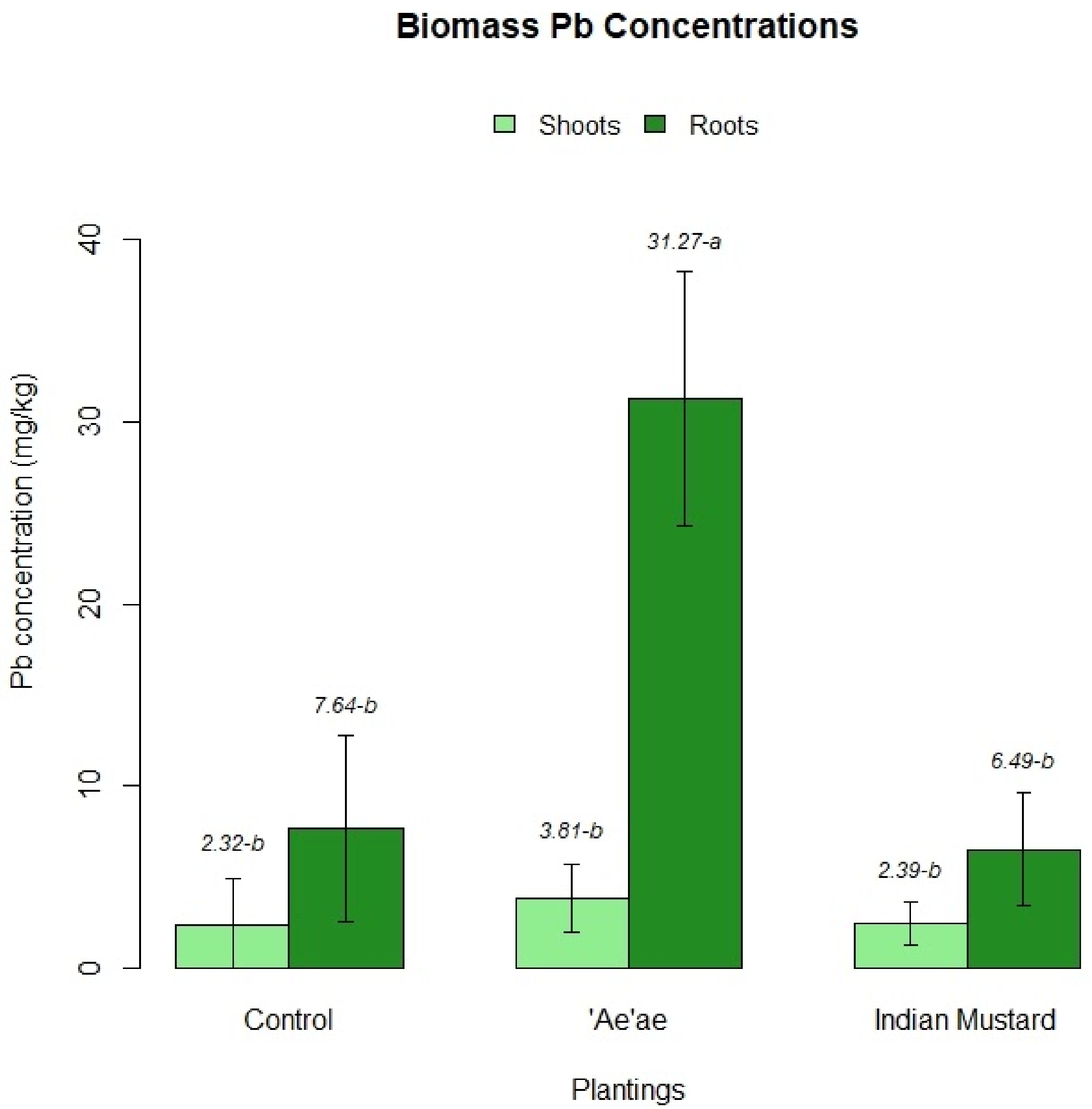

Figure 9. Average root (dark green) and shoot (light green) biomass Pb concentrations (mg/kg) of each phytoremediation planting with standard deviation bars. Letters next to mean values indicate significance according to the Tukey HSD test (a = $P < 0.001$, b = $P > 0.05$). ‘Ae‘ae roots contained significantly higher Pb concentration than any other plant biomass.

Table 1. Two way Analysis of Variance of biomass Pb concentrations among phytoremediation plantings (‘ae‘ae, Indian mustard, control plants) and biomass parts (roots or roots). Biomass Pb concentrations significantly differed among the combinations of plantings and and biomass parts (**bolded**).

	Sum of Squares	Df	F-value	*P*-value
Intercept	32.17	1	2.5513	0.12229
Plantings	8.45	2	0.3352	0.71821
Parts	84.90	1	6.7334	0.01535
Plantings:Parts	767.43	2	30.4318	**1.548e-07*** **
Residuals	327.83	26		

$*P < 0.05$, $**P < 0.01$, $***P < 0.001$

Plant Pb Uptake

Total Pb uptake significantly differed among phytoremediation plantings ($P < 0.001$; Table 2; Table A6). Indian mustard plantings, specifically, had a lower total Pb uptake ($P < 0.001$) than both the ‘ae‘ae and control plantings (Figure 10; Table A9). The ‘ae‘ae plantings had a slightly higher average total Pb uptake than control plantings, however, the total Pb uptake of the control plantings was highly variable. Note, most of the ‘ae‘ae Pb uptake occurred in the roots (70% roots), whereas Pb uptake in the Indian mustard (24% roots) and control plants (38% roots) occurred mostly in the shoots.

Table 2. One way Analysis of Variance of total Pb uptake among phytoremediation plantings (‘ae‘ae, Indian mustard, control plants). Total Pb uptake significantly differed among phytoremediation plantings (**bolded**).

	Sum of Squares	Df	F-value	*P*-value
Intercept	0.00082959	1	200.732	7.911E-15
Plantings	0.00044030	2	53.269	**1.343E-10*** **
Residuals	0.00012398	30		

$*P < 0.05$, $**P < 0.01$, $***P < 0.001$

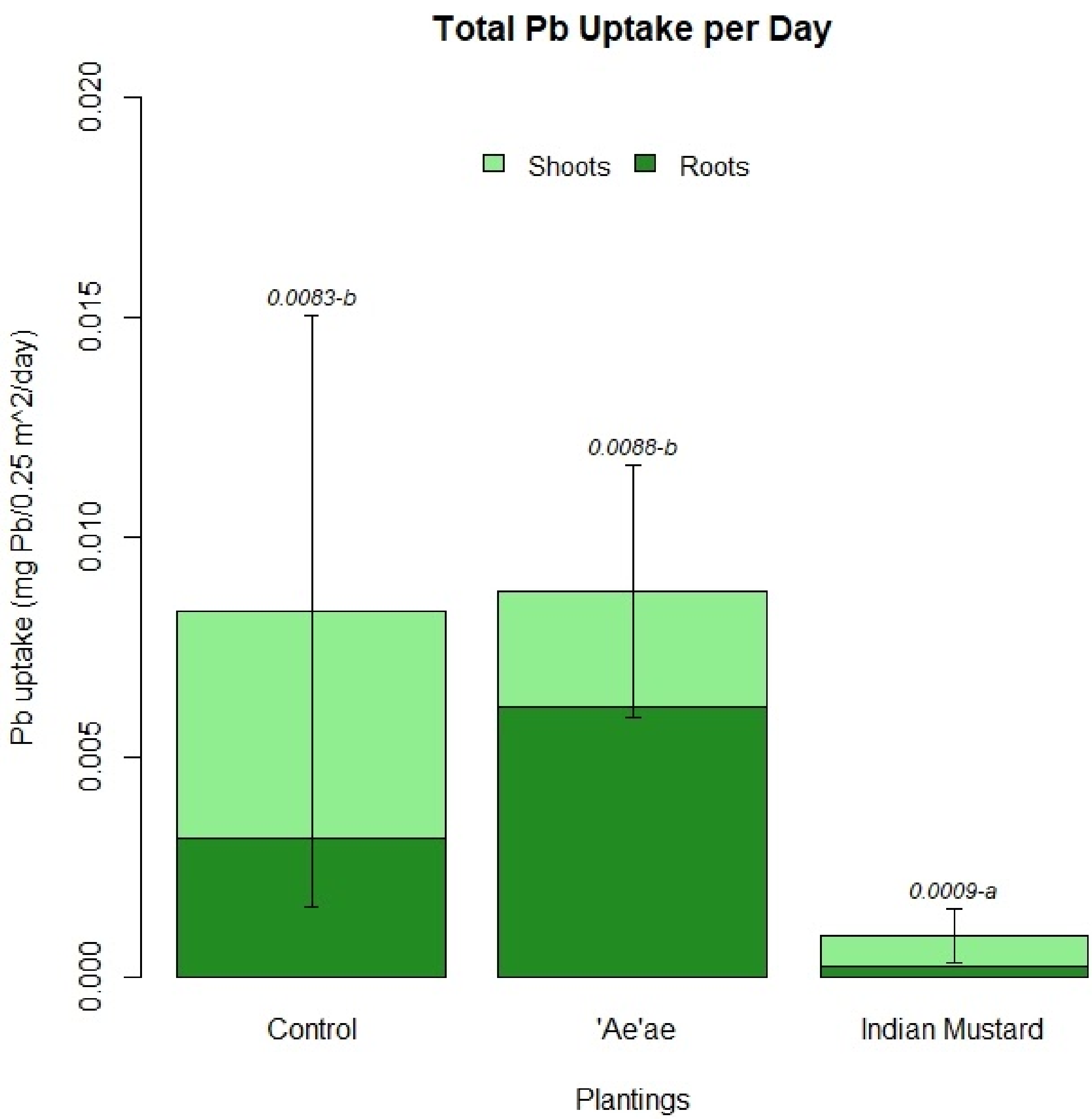

Figure 10. Average total Pb uptake (mg/0.25m^2/day) of each phytoremediation planting with standard deviation bars. Colors in bars indicate how much Pb uptake is occurring in roots (dark green) and shoots (light green). Letters next to mean values indicate significance according to the Tukey HSD test (a = *P* < 0.001, b = *P* > 0.05) Indian mustard plantings had a significantly lower total Pb uptake than both the ‘ae‘ae and control plantings.

Soil Pb Concentrations

Soil Pb concentrations did not differ between pre- and post-planting soil samples across any of the phytoremediation plantings (Figure 11). In fact, average post-planting soil Pb concentrations were higher than average pre-planting soil Pb concentrations.

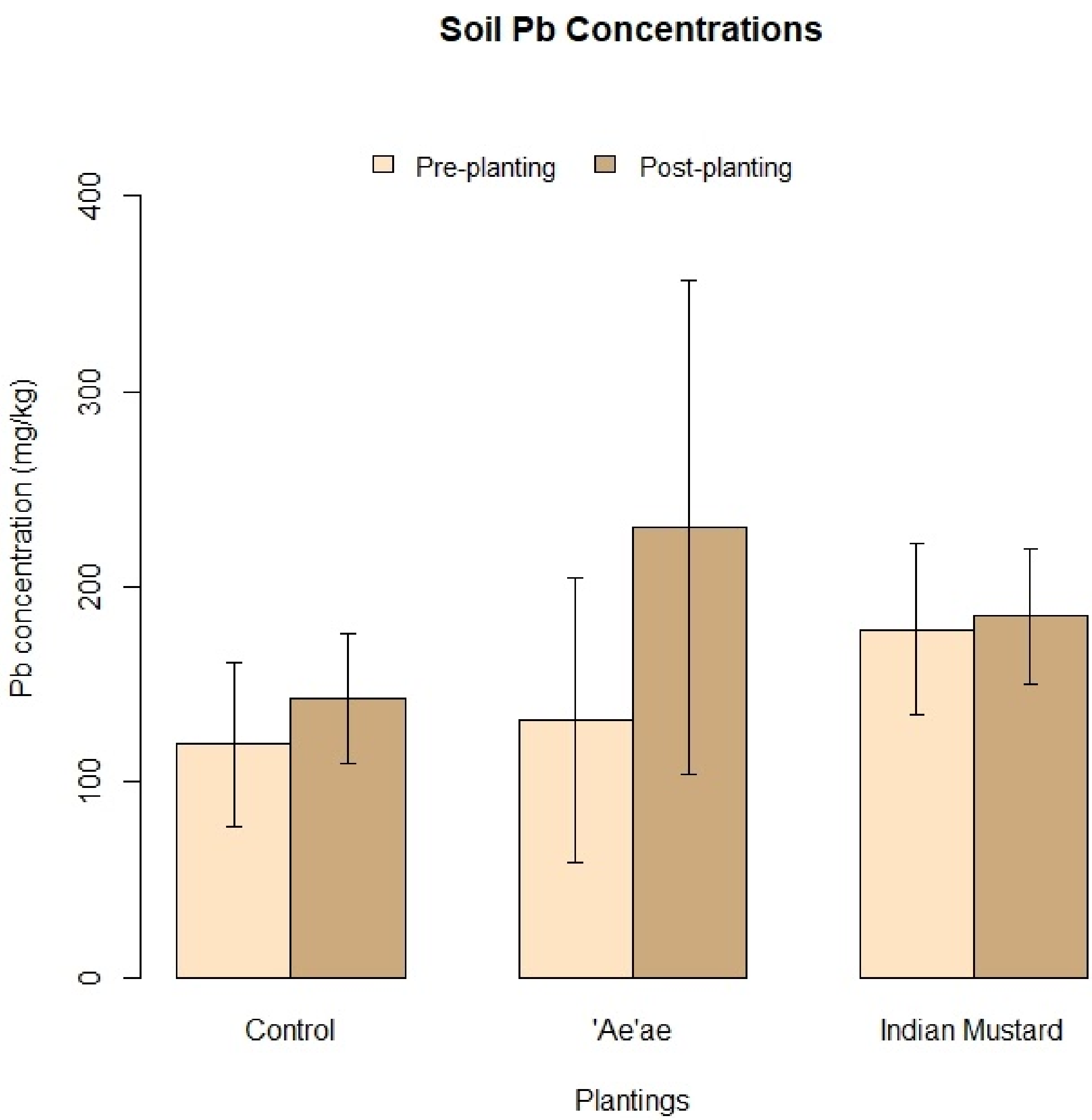

Figure 11. Average soil Pb concentrations (mg/kg) of lo‘i plots with standard deviation bars. There were no significant differences between pre- and post-planting soil Pb concentrations across all phytoremediation plantings.

Estimating Phytoremediation at Ulupō Site

Based on the mass of Pb in the Ulupō lo‘i plots (Table A10), all phytoremediation plantings would require ~1,000-120,000 rounds of phytoremediation (Figure 12; Table A10) to reduce lo‘i soil Pb concentrations to a safe level (0-75 mg/kg)[12]. This amount of phytoremediation rounds would equate to ~100-18,000 years of phytoremediation (Figure 13).

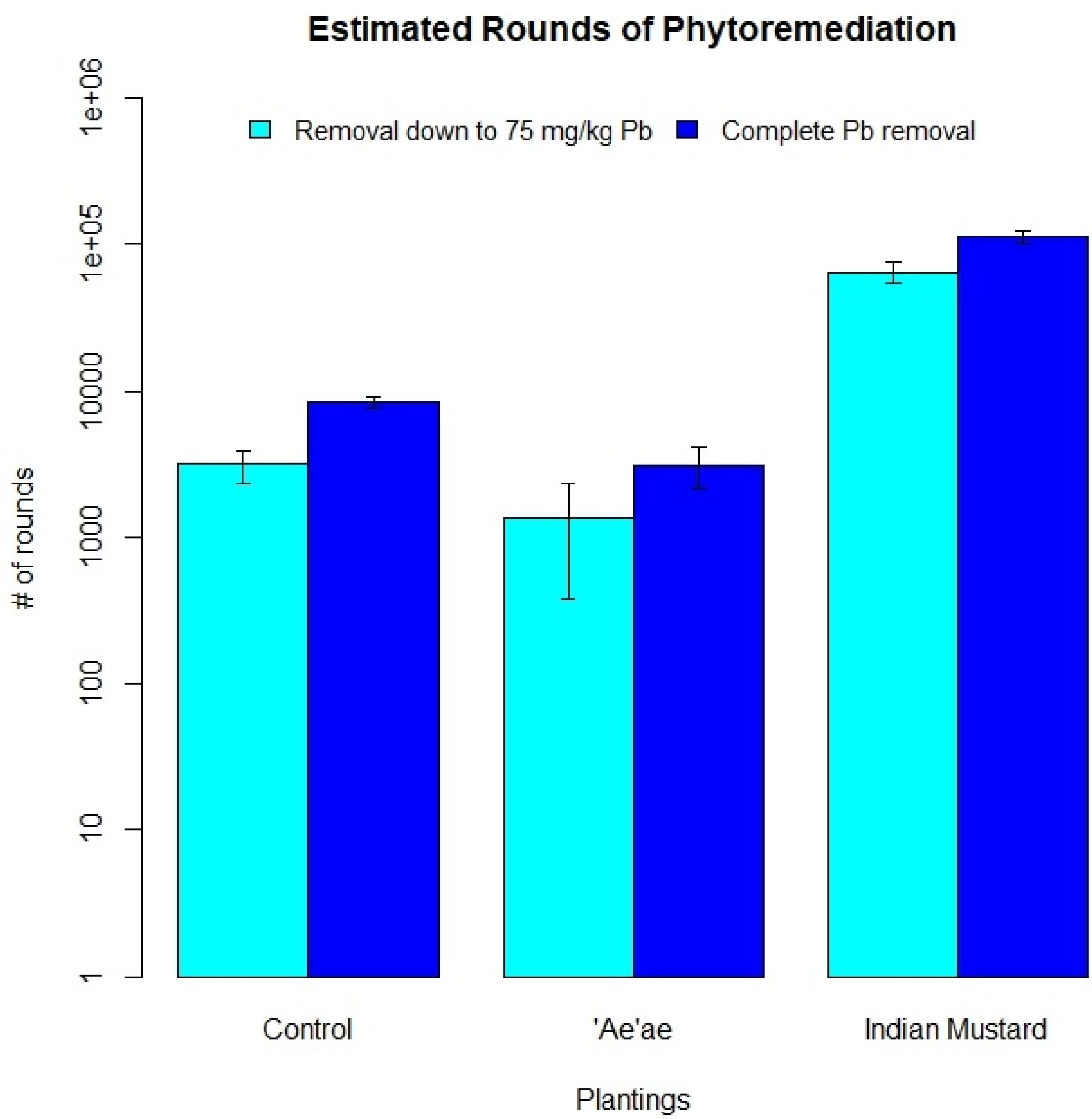

Figure 12. Average number of phytoremediation rounds needed to reduce lo‘i soil Pb concentrations to a safe level (0-75 mg/kg)[12] for each phytoremediation planting with standard deviation bars. Although ‘ae‘ae plants had the highest total Pb uptake, they still would need on average 1,000 rounds of phytoremediation to remediate the lo‘i soil.

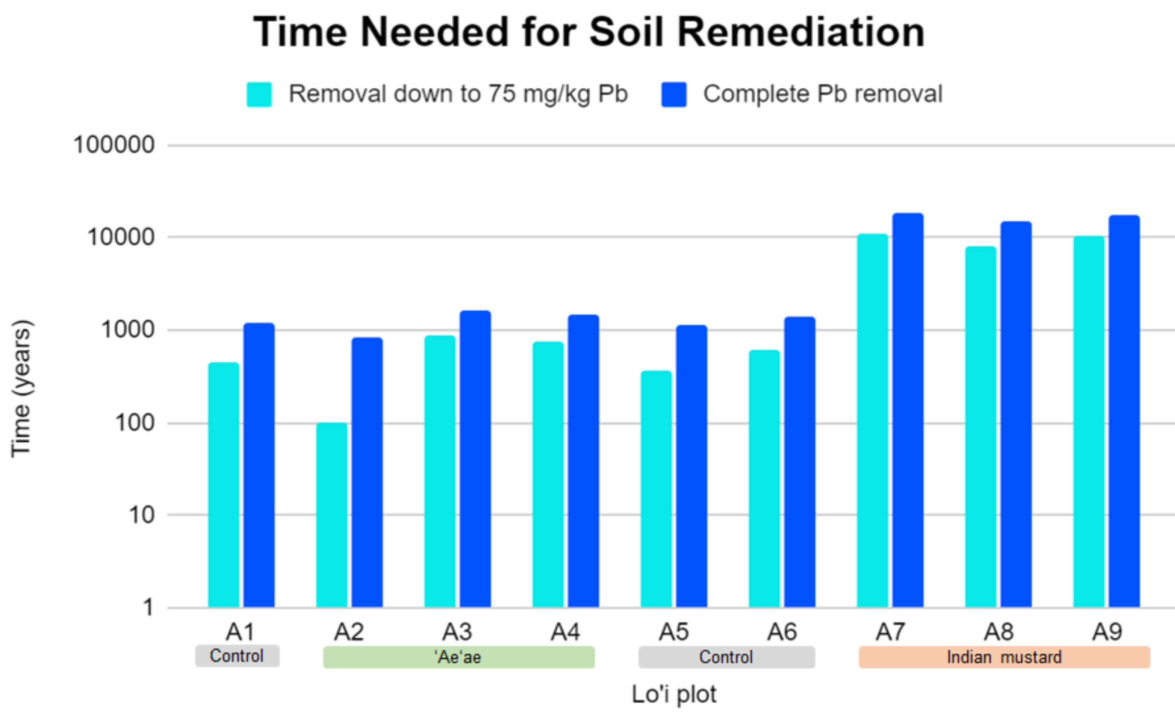

Figure 13. Time (years) needed to reduce soil Pb concentrations of each loʻi plot to a safe level (0-75 mg/kg)[12]. Assigned phytoremediation plantings are labeled below their respective loʻi plot. Loʻi plots at Ulupō would require ~100-18,000 years of remediation to reach a safe soil Pb level (0-75 mg/kg Pb).

Discussion

Comparing Phytoremediation Plantings

Overall, ‘ae‘ae plantings performed much better than the Indian mustard plantings. ‘Ae‘ae roots not only contained the highest Pb concentration, but ‘ae‘ae Pb uptake was significantly higher than Indian mustard Pb uptake. Couple reasons could account for this. First, ‘ae‘ae plants naturally thrive in lo‘i conditions, so their unhindered growth may have allowed for more Pb accumulation. Second, the flooded soil conditions specific for ‘ae‘ae growth may have led to a higher redox potential than the naturally irrigated soil conditions specific for Indian mustard plantings. Thus, Pb may have been more mobile and available for ‘ae‘ae uptake. Lastly, Indian mustard plantings struggled to grow in lo‘i conditions. Their low biomass growth likely hindered Pb accumulation, which consequently suggests that this plant is not a good candidate for phytoremediation in lo‘i systems. Perhaps the poor drainage of the lo‘i soil was a major barrier to Indian mustard growth.

For control plantings, assessing their ability to uptake Pb was difficult. Their Pb uptake was significantly higher than Indian mustard, however, their high variability of Pb uptake made comparisons with ‘ae‘ae inconclusive. Perhaps analyzing a mix of weedy species for Pb uptake, rather than individual weedy species led to this high variability. Moreover, the fine roots of the weedy species may have allowed for unwanted soil particles to cling to their roots, which would have also affected Pb uptake measurements.

Compared to other studies, both Indian mustard and ‘ae‘ae had relatively low Pb concentrations. For example, Indian mustard Pb concentrations were10 fold lower than what Salido et al. (2003) measured after growing Indian mustard in naturally contaminated soil (Pb concentration = 338 mg/kg)[20]. Also, ‘ae‘ae root and shoot Pb concentrations were 18 fold and 36 fold lower respectively, than what Sinha (1999) measured after growing ‘ae‘ae in artificially contaminated soil (using a 3μM Pb solution)[25]. Such discrepancies in Pb concentrations suggest a couple things. First, differences in experimental design (*in situ* vs. *ex situ*) may greatly affect plant growth and Pb uptake. A great example of this is how the Indian mustard growth and Pb uptake was likely hindered by lo‘i conditions, whereas Indian mustard growth and Pb uptake in Salido et al. (2003) was subject to ideal laboratory conditions. Second, the discrepancies also suggest that Pb in the lo‘i soil was much more immobile and unavailable for plant uptake. Compared to Sinha (1999) especially, all the Pb in their soil was already in a soluble and bioavailable form. Pb in real world environments, however, are mostly immobile[21].

Roots vs Shoots

Across all phytoremediation plantings, root biomass contained higher Pb concentrations than shoot biomass. This result may be attributed to unwanted soil particles clinging to the roots, however, this observed partitioning follows previous studies[21,22,25]. Interestingly, only ‘ae‘ae plants had Pb uptake occur mostly in roots rather than shoots. Sinha (1999) suggests that metal detoxifying substances (e.g., glutathione and ascorbate) found naturally in ‘ae‘ae roots can prevent translocation of Pb from roots to shoots. Thus, the mechanisms behind ‘ae‘ae’s high tolerance of heavy metals may be a barrier for Pb uptake in their shoots. Consequently, farmers implementing ‘ae‘ae for phytoextraction should focus on the removal of roots, which is much more tedious and time consuming than removing shoots.

Is Phytoremediation Applicable?

Although ‘ae‘ae plants appeared to be the better candidate for phytoremediation, ultimately, none of the tested plantings were effective at remediating Pb contaminated lo‘i soils on a reasonable timeframe. Soil Pb concentrations did not decrease after plantings, indicating one phytoremediation round had negligible effect. While soil Pb concentrations may have also been affected by differences in sampling methods, high spatial variability, and low sampling intensity, the likely reason is that Pb in the lo‘i soil was mostly immobile for plant uptake. Furthermore, the estimated amount of phytoremediation rounds (~1,000-120,000) equating to 100-12,000 years of remediation time indicates that implementing phytoremediation alone is clearly impractical. Selecting other plant species or testing alternative remediation methods will be needed to address Pb contamination of lo‘i soils.

Future Studies

There are several alternative methods worth testing. First, the addition of chelating agents to contaminated soils may significantly improve phytoextraction. The chelating agent EDTA (Ethylene diamine tetra-acetate), in particular, has shown to increase the bioavailability of Pb by 2800 fold with respect to a control soil (no EDTA)[22]. Since Pb bioavailability was a major challenge in this study, testing chelating agents in addition to phytoremediation would be valuable. EDTA has also shown to increase translocation of Pb from roots to shoots up to 120 times higher[21], which further enhances phytoremediation efforts. Careful considerations need to be taken though when applying chelating agents to avoid harmful leaching into downstream waterways[30].

Another potential investigation is cultivating microbial organisms to increase the bioavailability of Pb in the soil. Since certain microbes are capable of transforming heavy metals into nontoxic, mobile forms[14], perhaps deploying a combination of microbes and plants to remediate lo‘i soils can improve remediation effectiveness. This aspect of phytoremediation is still relatively new[14], so the effectiveness of this method is unknown.

Lastly, while the most efficient method of soil remediation is the physical removal of contaminated soil, it is often the most expensive[17]. Thus, an alternative is using a hybrid approach of removing the most contaminated soil (e.g. top 10 cm), and then applying phytoremediation plants. ‘Ae‘ae plants can be utilized since it performed the best throughout this study. Moreover, implementing a native plant for phytoremediation is especially significant for a place like Hawai‘i, where invasive species are a major environmental problem.

Conclusion

Lo‘i agriculture is one of the most important methods of traditional food cultivation in Hawai‘i, yet soil Pb contamination threatens the restoration of this important practice. In this study, three phytoremediation plantings grown in a Pb contaminated lo‘i site were evaluated for their effectiveness to phytoextract Pb from lo‘i soil. Although the native ‘ae‘ae plant accumulated the most Pb, applying ‘ae‘ae plantings alone would not be practical to remediate the lo‘i soil to a safe level. Thus, alternative methods of soil remediation should be considered in future studies.

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Appendix

Figure A1. Indian mustard plantings. (a) Indian mustard seeds. (b) Evenly hand dispersing 5,000 Indian mustard seeds per lo‘i. (c) Indian mustard plants after 5 weeks of growth.

Figure A2. ‘Ae‘ae plantings. (a) Trays of ‘ae‘ae plants. (b) Fragments of ‘ae‘ae separated from tray and ready for planting (~30 lbs of ‘ae‘ae per lo‘i). (c) Evenly hand dispersing ‘ae‘ae fragments into lo‘i. (d) ‘Ae‘ae plants after four months of growth.

Table A1. Time (days & years) needed to complete one round of phytoremediation for each of the phytoremediation plantings.

Planting	Time of one phytoremediation round (days)	Time of one phytoremediation round (years)
Control	56	0.15
Ae'ae	152	0.42
Indian Mustard	56	0.15

Table A2. Average (AVG) and standard deviation (STD) values of biomass growth, biomass Pb concentration, and Pb uptake for each of the phytoremediation plantings.

Planting	Part	AVG Biomass Growth ($kg/0.25m^2/day$)	STD Biomass Growth ($kg/0.25m^2/day$)	AVG Pb Conc. (mg/kg)	STD Pb Conc. (mg/kg)	AVG Pb Uptake (mg $Pb/0.25m^2/day$)	STD Pb Uptake (mg $Pb/0.25m^2/day$)
Control	shoots	0.00222585	0.000646876	2.3155	2.607112	**0.005153953**	**0.005993227**
Control	roots	0.00041395	0.000293768	7.635333	5.129087	**0.003160638**	**0.003088529**
‘Ae‘ae	shoots	0.00068562	0.00023503	3.806667	1.871221	**0.002609911**	**0.0015641**
‘Ae‘ae	roots	0.00019684	0.000006257	31.266667	7.021633	**0.006154395**	**0.002395239**
Indian Mustard	shoots	0.00029468	0.000201697	2.393333	1.203124	**0.000705268**	**0.000598935**
Indian Mustard	roots	0.00003354	0.000025327	6.492	3.120909	**0.000217725**	**0.000194911**

Table A3. Test for normal distribution (Shapiro-Wilk Test) among the biomass Pb concentration dataset. A *P*-value greater than 0.01 indicates that the dataset is normally distributed.

W-value	*P*-value
0.95439	0.1919

Table A4. Test for homogeneity (Levene’s Test) among the biomass Pb concentration dataset. A *P*-value greater than 0.01 indicates that the dataset is homogeneous.

	Df	F-value	*P*-value
Group	5	2.3551	0.06862

Table A5. Tukey HSD Test of biomass Pb concentrations among the combinations of phytoremediation plantings and biomass parts. The ‘ae‘ae roots contained significantly higher Pb concentrations than any other plant biomass. Note, only significant differences between combinations are listed ($P < 0.001$).

Interaction	Difference	Lower	Upper	*P*-value
‘Ae‘ae : Roots - Control : Shoots	28.95116667	21.2366050	36.665728	**0.0000000***
‘Ae‘ae : Roots - Control : Roots	23.63133333	15.9167717	31.345895	**0.0000000***
‘Ae‘ae : Roots - ‘Ae‘ae : Shoots	27.46000000	19.7454384	35.174562	**0.0000000***
‘Ae‘ae : Roots - Indian Mustard : Shoots	28.87333333	21.1587717	36.587895	**0.0000000***
Indian Mustard : Roots - ‘Ae‘ae : Roots	-24.77466667	-32.7422317	-16.807102	**0.0000000***

*$P < 0.001$

Table A6. Total Pb uptake of each phytoremediation planting. Total Pb uptake was calculated by adding the belowground and aboveground uptake values for each planting.

Planting	AVG Total Pb uptake (mg Pb/0.25m^2/day)	STD Total Pb uptake (mg Pb/0.25m^2/day)
Control	0.00831459	0.00674224
Ae'ae	0.00876431	0.0028607
Indian Mustard	0.00092299	0.00062985

Table A7. Test for normal distribution (Shapiro-Wilk Test) among total Pb uptake dataset. A *P*-value greater than 0.01 indicates that the dataset is normally distributed.

W-value	*P*-value
0.95028	0.1356

Table A8. Test for homogeneity (Levene's Test) among the total Pb uptake dataset. A *P*-value greater than 0.01 indicates that the dataset is homogeneous.

	Df	F-value	*P*-value
Group	2	4.7516	0.01612

Table A9. Tukey HSD Test of total Pb uptake among the phytoremediation plantings. The Indian mustard plants had a significantly lower Pb uptake than any other planting. ($P < 0.001$).

Interaction	Difference	Lower	Upper	*P*-value
ʻAeʻae - Control	0.0004467154	-0.001760252	0.002659683	0.871065
Indian mustard - Control	-0.0073915957	-0.009437628	-0.005345564	**0.0000000***
Indian mustard - ʻAeʻae	-0.0078413111	-0.010051278	-0.005631344	**0.0000000***

*$P < 0.001$

Table A10. Data of each loʻi plot including the amount of phytoremediation rounds needed to reduce loʻi soil Pb concentrations to a safe level (0-75 mg/kg Pb, HDOH 2017).

Loʻi plot	Planting	Area (m^2)	Volume of soil (m^3)	Mass of Soil (kg)	AVG Pb Conc. (mg/kg)	STD Pb Conc. (mg/kg)	Mass of Pb in Loʻi (mg)	Total Plant Uptake (mg Pb)	**# of Phytorem. Rounds (complete Pb removal)**	**# of Phytorem. Rounds (up to 75 mg/kg Pb)**
A1	Control	41.499	4.1499	5394.85	117.06	31.7302	631521	77.2903	**8171**	**2936**
A2	ʻAeʻae	42.840	4.2840	5569.25	85.3333	67.0717	475242	237.0978	**2004**	**243**
A3	ʻAeʻae	54.560	5.4560	7092.81	162.283	70.4690	1151045	301.9599	**3812**	**2050**
A4	ʻAeʻae	36.910	3.6910	4798.35	150.64	66.2914	722823	204.2785	**3538**	**1777**
A5	Control	23.870	2.3870	3103.16	110.72	56.0949	343572	44.4580	**7728**	**2493**
A6	Control	47.283	4.7283	6146.79	132.52	36.8137	814572	88.0631	**9250**	**4015**
A7	Indian mustard	36.646	3.6646	4763.93	191.78	49.6659	913626	7.5765	**120587**	**73429**
A8	Indian mustard	16.381	1.6381	2129.49	159.145	45.1481	338898	3.3867	**100067**	**52909**
A9	Indian mustard	18.150	1.8150	2359.44	184.727	32.5426	435853	3.7524	**116152**	**68994**

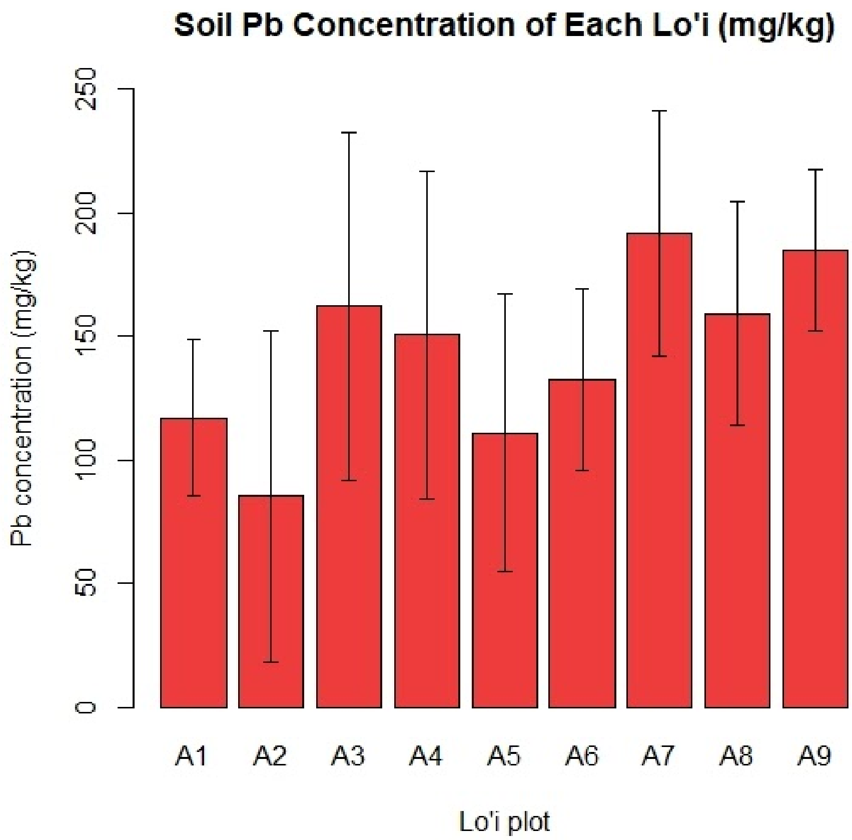

Figure A3. Pre-planting soil Pb concentration of each lo‘i plot (mg/kg). Error bars indicate standard deviation. Average soil Pb concentrations of the lo‘i plots ranged from 85-191 mg/kg Pb, with an overall average 143.8 ± 52.6 mg/kg Pb.